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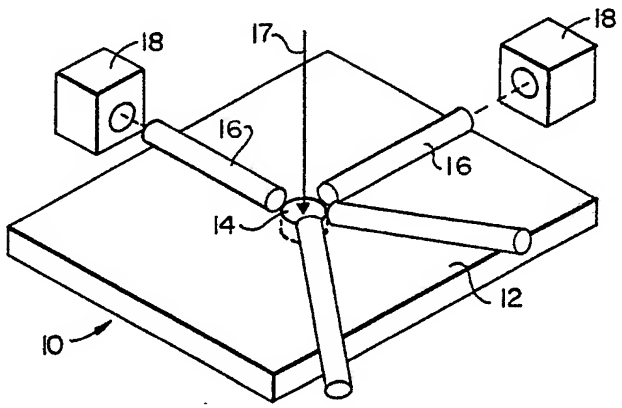

(57) Abstract

An optical system (10) for use in flow cytometers comprises a plurality of waveguides (16) supported on an orifice plate (12) used to define the flow stream (17). The waveguides (16) terminate at an aperture (14) in the orifice plate (12) through which the flow stream (17) is directed. The ends of the waveguides (16) distal from the aperture (14) are variously coupled to illumination sources (18) and detector (18). In a preferred embodiment, the waveguides (16) are optical fibers bonded to the orifice plate (12). In another preferred embodiment, the orifice plate (12) serves as the substrate for an integrated optics device, the waveguides (16) being provided by appropriate implantation.

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OPTICAL SYSTEMS FOR FLOW CYTOMETERS

BACKGROUND OF THE INVENTION

This invention pertains to the flow cytometers and more particularly to such devices wherein a number of different optical measurements are simultaneously made.

Optical flow cytometers, in which cells having predetermined optical characteristics are detected, counted, and/or sorted as they pass an observing station, are well known. In such systems, the individual cells are illuminated and observed, preferably simultaneously, by one or more optical channels. Observations may be made over one or more wavelength bands and at a variety of angles to the incident illumination. Cells are counted or sorted according to the observed values of the the appropriate optical properties (e.g., color, scattering characteristics, fluorescent signature, optical density, or the like). In the more sophisticated flow cytophotometers, a variety of parameters are observed and the cells are classified for counting or sorting according to the correlation of these various parameters.

Generally, only a very restricted volume of the sample is observed, both in order to insure

measurements of single, rather than random groups of cells, and to improve the photometric accuracy of the measurements. In a typical system, lenses (usually microscope objectives) are used to focus the illuminating radiation onto an observation region near the center of the flow stream and to collect the transmitted, scattered, and/or emitted radiation from the region.

While satisfactory, such an arrangement suffers from a number of drawbacks. For instance, mechanical considerations dictate that only a limited number of lenses (typically four) can be mounted at any one station along the flow stream. A greater number of microscope objectives focussed on the same region is difficult to accommodate, since the mechanical structures supporting the individual objectives typically interfere with one another. This limits the number of observations of a given cell that may be made simultaneously, and thereby complicates multiparameter systems, which might therefore require the correlation of observations taken seriatim.

Similar mechanical considerations constrain the spacing of multiple optical stations along the flow stream, or the similar spacing of electrical or fluidic cell sorting apparatus or measuring devices from an optical station. Beyond affecting the size of the apparatus, the spacing of the various elements

along the flow stream impacts upon various operational system parameters, such as the velocity constancy required to insure proper assignment of measurements from different stations to the same cell for a given counting rate.

Additionally, such designs require the precision alignment of a number of microscope objectives. This is difficult to achieve and to maintain over extended periods of time.

Another consideration is one of cost. A typical multi-parameter flow cytophotometer incorporates a number of high-quality microscope objectives, together with their precision alignment mechanisms, impacting costs of parts, assembly, and routine maintenance.

OBJECTS OF THE INVENTION

Accordingly, an object of the present invention is to provide an optical apparatus for use in flow cytometers wherein a maximum number of optical channels may be situated in one plane transverse to the flow stream.

Another object of the present invention is to provide an optical apparatus for use in flow cytometers that permits close spacing of a number of observing stations, or observing stations and ancillary systems, along the flow stream.

A further object of the present invention is to provide an optical apparatus for use in flow cytometers that insures alignment of a number of associated optical paths without the requirement for time-consuming alignment procedures or costly precision alignment devices.

Yet another object of the present invention is to provide an optical system for multi-parameter flow cytophotometers which is relatively inexpensive.

BRIEF DESCRIPTION OF THE INVENTION

These and other objects are met in the present invention of an optical system for flow cytometers wherein an orifice plate, used to define the flow stream, serves as a substrate to support a plurality of optical waveguides. The waveguides terminate at the aperture in the orifice plate through which the flow stream passes. The ends of the waveguides distal from the aperture are variously coupled to illumination sources and detectors. In a preferred embodiment, the waveguides are optical fibers bonded to the orifice plate. In another preferred embodiment, the orifice plate serves as the substrate for an integrated optics device, the waveguides being provided by appropriate implantation.

Inasmuch as the orfice plate serves as the

mechanical mounting for the waveguides, and the waveguides are bonded thereto, the mechanical constraints of normal optical-mechanical structures are avoided. Consequently, a maximum number of coplanar optical channels radiating from a single center can be provided. Further, as the alignment is built into the system, elaborate precision alignment devices are not required, nor is there any need for time-consuming alignment procedures. It will also be recognized that the aperture plate and waveguide structure of the present invention may be fabricated by relatively straight forward procedures, resulting in a relatively inexpensive optical system.

Other objects of the invention will in part be obvious and will in part appear hereinafter. The invention accordingly comprises the apparatus possessing the construction, combination of elements, and arrangement of parts which are exemplified in the following detailed disclosure, and the scope of the application of which will be indicated in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the nature and objects of the present invention, reference should be had to the following detailed description taken in connection with the accompanying drawings wherein:

Fig. 1 is a perspective view of a preferred embodiment of the present invention, as seen from an angle oblique to the direction of the flow stream of a flow cytometer;

Fig. 2 is a fragmentary plan view of the embodiment of Fig. 1, viewed parallel to the direction of the flow stream; and

Fig. 3 is a fragmentary sectional view taken along the lines 3-3 of Fig. 2, showing the embodiment of Fig. 1 and the immediately adjacent structure of a flow cytometer.

DETAILED DESCRIPTION

Referring to FIG. 1, there may be seen a a preferred embodiment of the present invention. The fluid stream of a flow cytometer (not shown) is made to flow past one or more observing stations 10, as by gravity or pump means well known in the art. Each observing station 10 comprises an orifice plate 12, preferably in the form of a thin, flat plate provided with a small, centrally located, circular aperture 14, and a plurality of optical waveguides 16. Orifice plate 12 is installed in a cytometer so that the plane of the plate is transverse to the direction of flow of the fluid stream, which, it will be understood, is directed at the plate so as to pass through aperture

14, as schematically indicated by arrow 17.

In greater detail, orifice plate 12 is preferably of a material chosen to be impermeable to and non-reactive with the fluids of interest. Typical materials of construction of orifice plate 12 include, but are not limited to, glass, fused silica, saphire, polypropylene, polyolefin, nylon, or the like. It will be understood that, if necessary, orifice plate 12 may be supplied with a coating to provide physical isolation between it and the flow stream or optical isolation between it and waveguides 16, if necessary. The overall dimensions of orifice plate 12 are not particularly critical, and are chosen as convenient. Typical dimensions are a few centimeters square by one millimeter thick, although other dimensions may be accommodated.

In a preferred embodiment, aperture 14 is a substantially right circular cylindrical bore, substantially normal to and penetrating through orifice plate 12. However, it will be appreciated that for various applications the bore forming the aperture may be a right regular prism or a right circular conic frustum. In the latter case, the aperture will normally be placed in the flow stream with the smaller diameter base of the frustum upstream of the larger. The dimensions of aperture 14 are set primarily by considerations of the cells to be

observed, and for most cases will be typically between about 20 micrometers and about 200 micrometers in diameter. It will be understood that aperture 14 is dimensioned to permit free, undisturbed passage of cells flowing substantially along the axis of the aperture, and to dispose the cells a sufficient distance from the walls of the aperture to permit adequate illumination and viewing, as will become apparent. Aperture 14 may be formed in orifice plate 12 by a variety of means, such as micromachining, chemical milling, laser drilling, or the like. As already noted, aperture 14 is preferably centrally situated in orifice plate 12.

Optical waveguides 16 may take the form of individual optical fibers attached to orifice plate 12, or may be portions of an integrated optical device implanted or otherwise formed on or in the orifice plate, as by casting, ion implantation, vapor deposition, or the like. A preferred embodiment employs cladded optical fibers. As may be seen by reference to FIGs 1 and 2, waveguides 16 are arranged substantially in a single plane (as, for instance, a surface of orifice plate 12) so as to extend radially from aperture 14. An end of each waveguide terminates at aperture 14 such that the optical axis of the waveguide is substantially normal to the axis of the aperture (and the flow stream). In a preferred

embodiment, up to six optical fibers, each 100 micrometers in diameter, may be so disposed about a 200 micrometer diameter aperture (FIG. 2); obviously, a larger number of waveguides of smaller diameter could be disposed about the same size aperture.

A particularly convenient method for insuring that the individual waveguides terminate at aperture 14 is to initially fabricate the waveguide and orifice plate structure without an aperture. Aperture 14 is then drilled through the center of an abutted fan of waveguides, as by laser drilling. This approach is particularly suited to the construction of a pair of waveguides intended for transmission measurements: drilling through a single straight waveguide assures the desired 180 degree alignment of the resulting pair of waveguides.

The ends of the waveguides distal from aperture 14 are coupled to various optical sources and detectors, schematically represented by index numbers 18, in accordance with the measurements to be undertaken, as will be understood by those skilled in the art.

Waveguides 16 are variously used for illumination and collection, and it will be understood that the form of the waveguide may be varied accordingly. Thus, single-mode optical fibers having graded index sheaths may be used as desired for illumination, in order to provide a narrow illuminating beam. Commercially

available fibers having an outer diameter of about 100 micrometers may be used with visible laser sources to provide a beam having a beam diameter at the fiber face of about 5 micrometers, spreading to some 70 micrometers as it passes across a 200 micrometer orifice. For purposes of collecting radiation emitted, transmitted, or scattered by the passing cells, multi-mode fibers may be advantageous because of their larger optical throughput.

The exact configuration of waveguides 16 depends on the optical parameters to be observed, as is well known in the art of cytophotometry. Thus, transmission measurements require illuminating and collecting waveguides to be in line with one another. Fluorescent measurements may be made with illuminating and collecting waveguides not in line, the difference in viewing angle providing some isolation of the illuminating and fluorescent radiation. The observation of scattering signatures requires collection at a number of angles to the illumination direction.

The axes of waveguides 16 preferably should be disposed substantially radially to aperture 14 proximate to the aperture, in order to illuminate and view the core of the flow stream passing through the aperture. However, there is no general requirement that the radial orientation of the waveguides be

maintained distal from the aperture, and the waveguides may be curved, as necessary, in order to more conveniently accommodate sources and detectors.

The optical system of the present invention has a number of advantages. Inasmuch as the orfice plate serves as the mechanical mounting for the waveguides, and the waveguides are bonded thereto, the mechanical constraints of normal optical-mechanical structures are avoided. Consequently, a maximum number of coplanar optical channels radiating from a single center can be provided. Further, as the alignment is built into the system, elaborate precision alignment devices are not required, nor is there any need for time-consuming alignment procedures. It will also be recognized that the aperture plate and waveguide structure of the present invention may be fabricated by relatively straightforward procedures, resulting in a relatively inexpensive optical system.

As already indicated, waveguides 16 might be other optical devices than cladded fibers. For instance, waveguides 16 might be integrated optical waveguides implanted in or on orifice plate 12 as by ion beam implantation, vapor deposition, or the like. Then too, fiber waveguides may be recessed into the surface of orifice plate 12, rather than attached to the surface. The use of cladded fibers cemented to the surface merely allows ready assembly of individual

custom-made observing stations 10, since the various masks and processing steps normally required for the production of integrated optical devices are not required. Fibers may be simply cemented, as with epoxy adhesive, to orifice plate 12 in the desired configuration. For such applications, the use of cladded fibers makes it unnecessary to otherwise optically isolate either the orifice plate from the waveguides or closely adjacent waveguides from one another. It will be appreciated, however, that uncladded fibers might be used, in which case the orifice plate would have to be optically isolated from the waveguides, as by a suitable coating or an appropriate choice of materials, and the waveguides either spaced well apart or otherwise isolated from one another in order to prevent cross-talk.

An integrated-optic observing station, similar to that described for the case of a fiber optic station, is a preferred embodiment for quantity production of observing stations, as such devices may be quantity produced relatively cheaply once the initial tooling is available. In such apparatus, the full scope of integrated optics may be used to provide additional optical elements, such as filters, dispersive devices, and the like, on the substrate provided by orifice plate 12. Such an approach for the fabrication of observing stations also offers the possibility of

integrating sources (such as LEDs) and detectors (such as photodiodes), and electronics into the manufacturing process, building, in effect, an integrated chip containing source, optics, detectors, and electronics.

It will also be appreciated that waveguides 16 may be similarly disposed about aperture 14 on both surfaces of an orifice plate 12, as shown in FIG. 3. In this way, a pair of closely spaced oberving stations may be disposed along the flow stream.

FIG. 3 also indicates the manner in which one or more observing stations 10 are positioned in the flow stream of a cytometer. As well known in the art, the cells to be observed are entrained in a core flow stream 20 that is centered in a sheath fluid 22 conducted through a channel 24. Core stream 20 is injected into the center of channel 24 by an injection channel 26 that terminates a short distance up stream from the observing station and in line with aperture 14. Core stream 20 necks down as it passes through aperture 14, and the cells entrained in the core flow are directed substantially centrally through the aperture. As may also be seen in FIG. 3, a plurality of aperture plates may be disposed in closely spaced arrangement along the direction of flow of the fluid stream.

It will be appreciated that orifice plate 12 of

observation station 10 may also serve as the substrate for other components of a flow cytometer. Thus, the surfaces of the orifice plate or of aperture 14 might be made electrically conductive, to serve as an electrical counting or measuring device or the charging electrode or ground ring of an electrostatic cell sorting mechanism. Then, too, the orifice plate may form the substrate of an acoustic transducer to serve as the droplet generator of a sorting mechanism.

It will also be understood that, although described in terms of use with a flow cytometer, the optical system of the present invention may be used to observe particulates other than cells in a flow stream.

Since these and certain other changes may be made in the above described apparatus without departing from the scope of the invention herein involved, it is intended thta all matter contained in the above description or shown in the accompanying drawings shall be interpreted in an illustrative and not in a limiting sense.

WHAT IS CLAIMED IS:

1. Apparatus for use in flow photometers wherein particulates included in a flow stream are classified and counted, said apparatus comprising, in combination:

a plate having an aperture extending therethrough and dimensioned and disposed for restricting the flow of said flow stream; and

a plurality of optical waveguides supported by said plate, said waveguides each terminating at said aperture and extending radially therefrom, at least one of said waveguides being disposed to direct, when illuminated distal from said aperture, illumination along an axis transverse to said aperture, others of said waveguides being disposed to collect radiation from particulates illuminated in said aperture by said illumination, and transmit said radiation to locations distal from said aperture.

2. Apparatus as claimed in claim 1 wherein said waveguides are optical fibers bonded to the surface of said plate.

3. Apparatus as claimed in claim 2 wherein said at least one of said waveguides is configured to operate in a single mode.

4. Apparatus as claimed in claim 1 wherein said plate is the substrate of an integrated optics device and said waveguides are implanted thereon.

5. Apparatus as claimed in claim 1 wherein said waveguides are all disposed substantially in a single plane.

6. Apparatus as claimed in claim 1 wherein said waveguides are disposed in two sets, one on each of the opposite sides of said plate.

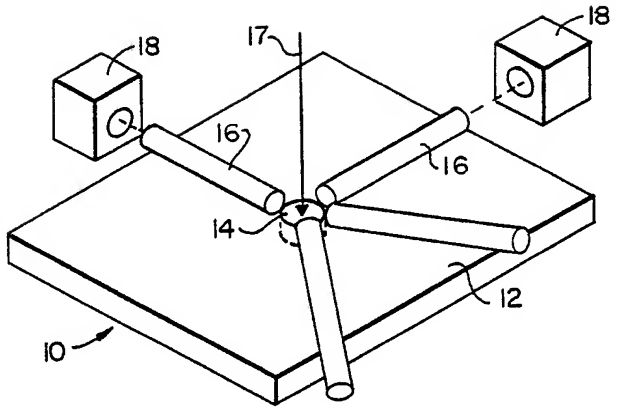

FIG. 1

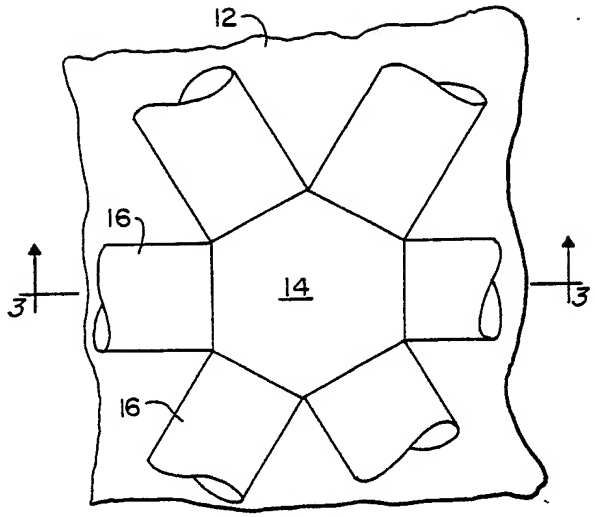

FIG. 2

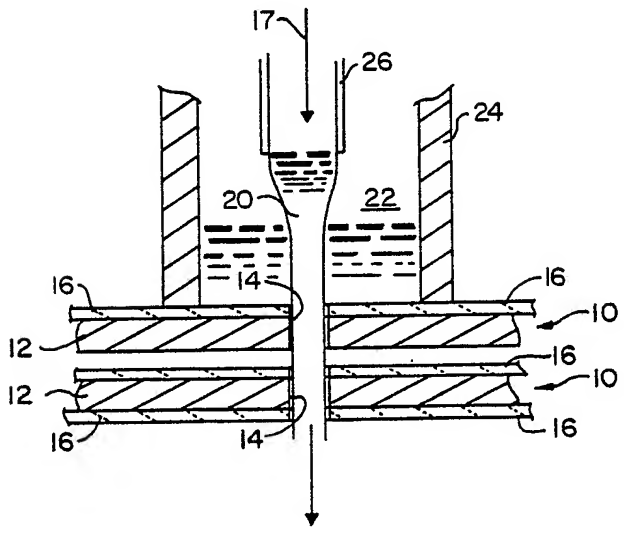

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/00989

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL. 3 G01N 21/05; G01N 33/48
U.S. CL. 356/73; 356/39

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	356/73,335,336,338,39,70 ; 377/10-12 ; 250/574,575,576,227,461.2; 324/71.4 ; 362/32; 350/96.10, 96.11, 96.12 ; 209/4

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	U S, A, 4,201,471 (Pitt et al), 06 May 1980	1-6
Y	U S, A, 3,924,951 (Dittrich), 09 December 1975	1-6
Y	U S, A, 4,079,404 (Comerford et al), 14 March 1978	1-6
Y	D E, A, 1,932,627 (Dittrich), 18 March 1971	1-6
Y	U S, A, 3,883,219 (Logan et al), 13 May 1975	2,4,5
Y	U S, A, 4,318,180 (Lundqvist et al), 02 March 1982	6
A	U S, A, 3,591,290 (Zinner et al), 06, July 1971	1

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
26 JUNE 1985	18 JUL 1985
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Robert Thompson ROBERT THOMPSON

Form PCT/ISA/210 (second sheet) (October 1981)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	U S, A, 4,348,107 (Leif), 07 September 1982	1
A	U S, A, 4,250,394 (O'Conner) 10 Feburary 1981	1
Y	N, IBM Technical Disclosure Bulletin, Vol. 20, No. 3, issued August 1980 (Armonk, New York), M. Johnson, "Stress Induced Waveguides in Thin-Film Transparent Rubbers", Page 1269.	2,3,4

DERWENT-ACC-NO: 1986-006928

DERWENT-WEEK: 198624

TITLE: Optical system for flow cytometer classifies and counts particles flowing using several optical fibre waveguides bonded to surface of plate

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PATENT-ASSIGNEE: SHAPIRO H M[SHAPI]

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PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
WO 8505680 A	December 19, 1985	EN
EP 183798 A	June 11, 1986	EN

DESIGNATED-STATES: JP AT BE CH DE FR GB IT LU NL SE AT BE CH DE FR GB IT LI LU NL SE

APPLICATION-DATA:

PUB-NO	APPL-DESCRIPTOR	APPL-NO	APPL-DATE
WO1985005680A	N/A	1985WO-US00989	April 28, 1985
EP 183798A	N/A	1985EP-902889	May 28, 1985

INT-CL-CURRENT:

TYPE	IPC DATE
CIPS	G01N15/14 20060101

ABSTRACTED-PUB-NO: WO 8505680 A

BASIC-ABSTRACT:

The system comprises in combustion a plate having an aperture extending through it and dimensional and disposed so as to restrict the flow of the flow stream. Several optical waveguides are supported by the plate. The waveguides each terminate at the aperture and extend radially from it. At least one of the waveguides is disposed to direct, when illuminated distally from the aperture, illumination along an axis transverse to the aperture. The other waveguides are disposed to catch radiation from particulates illuminated in the aperture by the illumination.

The light is then transmitted to distal locations away from the aperture. The waveguides are optical fibres bonded to the plate surface and one of the waveguides operates in a singlemode.

ADVANTAGE - Allows presence of number of microscope objectives.

TITLE-TERMS: OPTICAL SYSTEM FLOW CYTOMETRY CLASSIFY COUNT PARTICLE FIBRE WAVEGUIDE BOND SURFACE PLATE

DERWENT-CLASS: S03 S05 T05

EPI-CODES: S03-E04X; S03-E14H; S03-F05; S03-G02X; S05-C; T05-A02; T05-K;